CellPress

Neuron
Review

Disruption of RNA Metabolism in Neurological Diseases and Emerging Therapeutic Interventions

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https://doi.org/10.1016/j.neuron.2019.03.014

RNA binding proteins are critical to the maintenance of the transcriptome via controlled regulation of RNA processing and transport. Alterations of these proteins impact multiple steps of the RNA life cycle resulting in various molecular phenotypes such as aberrant RNA splicing, transport, and stability. Disruption of RNA binding proteins and widespread RNA processing defects are increasingly recognized as critical determinants of neurological diseases. Here, we describe distinct mechanisms by which the homeostasis of RNA binding proteins is compromised in neurological disorders through their reduced expression level, increased propensity to aggregate or sequestration by abnormal RNAs. These mechanisms all converge toward altered neuronal function highlighting the susceptibility of neurons to deleterious changes in RNA expression and the central role of RNA binding proteins in preserving neuronal integrity. Emerging therapeutic approaches to mitigate or reverse alterations of RNA binding proteins in neurological diseases are discussed.

During their life cycle, messenger RNAs (mRNAs) undergo extensive processing steps including splicing, polyadenylation, editing, transport, translation, and turnover. This elaborate process is highly dynamic and requires a complex interplay among RNA binding proteins (RBPs) to finely modulate co- and post-transcriptional processing of transcripts. RBPs bind RNA molecules at specific sequences or secondary structures in order to facilitate several steps of RNA processing, both in the nucleus and cytoplasm. Genome-wide approaches have provided major insights into the multiple ways RBPs influence the fate of their targets (Nussbacher et al., 2015). Indeed, RBPs are increasingly recognized as multifunctional proteins since a specific RBP may associate with different protein complexes to influence several processing steps of its RNA targets. It is therefore not surprising that altered interaction between an RBP and its targets can lead to severe pathological phenotypes. In fact, the weight of evidence supports that defects in RNA regulation play a crucial role in neurodevelopmental dysfunction and neurodegenerative diseases. The mechanisms by which RBPs are altered in neurological disorders are diverse, and in several cases the molecular details remain elusive. However, a few themes have emerged, including impaired RBP expression, cellular mislocalization and aggregation of RBPs, and sequestration of RBPs by transcripts and/or abnormal proteins with pathological repeat expansions (Figure 1; Table 1). Even small changes in RBP expression or activity may be amplified due to their broad impacts on expression, splicing, and translation of hundreds of RNA substrates, and their functional disruption can have global and significantly deleterious outcomes on the health of neurons. Here, we discuss the multiple ways by which perturbations of RNA homeostasis are associated with neurological diseases. The groundbreaking recognition of RBPs as major contributors to neurodegenerative diseases is illustrated by specific examples and descriptions of genome-wide studies that documented the impact of widespread RNA processing misregulation in disease. Methods to therapeutically restore the functions of RBPs are emerging and current strategies to combat RBP homeostasis perturbations are also discussed.

Reduced Expression of RBPs in Neurological Disorders

Neurological disorders may be induced by mutations reducing or silencing the expression level of RBPs (Figure 1A) as demonstrated for SMN1 in spinal muscular atrophy and FMRP in fragile X syndrome (FXS).

Altered SMN1 Expression in Spinal Muscular Atrophy

Spinal muscular atrophy (SMA), characterized by degeneration of lower motor neurons and severe muscular atrophy, has an incidence rate of ~1 in 6,000–10,000. This autosomal recessive disease is caused by loss-of-function mutations, homozygous deletions, and less frequently point mutations, in the survival of motor neuron 1 (SMN1) gene (Lefebvre et al., 1995). The closely related SMN2 gene generates only low levels of functional SMN protein. Indeed, a C > T difference in SMN2 results in the exclusion of exon 7 in 80%–90% of transcripts and the production of a truncated protein unable to efficiently compensate for loss of SMN1 (Kashima and Manley, 2003; Lorson et al., 1999). Notably, inefficient splicing of SMN2 exon 7 is markedly exacerbated in motor neurons, contributing at least partially to the vulnerability of motor neurons in SMA patients (Ruggiu et al., 2012). SMN is essential for the assembly of small nuclear ribonucleoproteins (snRNPs), achieved through self-oligomerization followed by interaction with small nuclear RNAs (snRNAs), gemins, and Sm proteins in the cytoplasm (Li et al., 2014; Liu et al., 1997; Pellizzoni et al., 1998; So et al., 2016). This complex is imported to

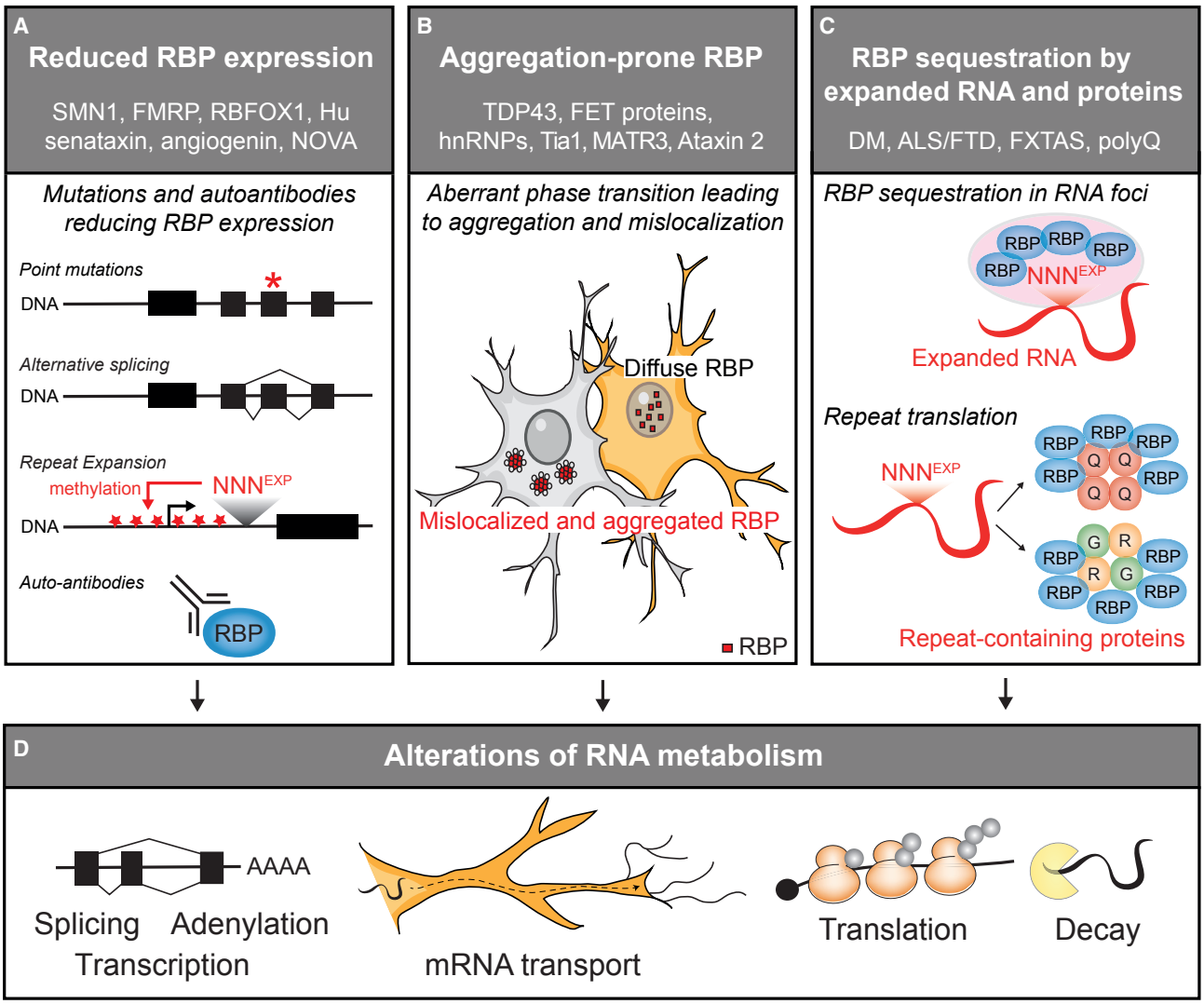

Figure 1. Mechanisms Leading to Disruption of RBPs in Neurodegenerative Diseases

(A) Expression of RBPs may be reduced by point mutations, abnormal splicing, aberrant repeat expansions that repress the RBP transcription, and neutralization by autoantibodies in paraneoplastic neurological syndromes.

(B) Abnormal phase transition of RBPs with low complexity domains leads to their aggregation and mislocalization with loss of function and/or gain of novel toxic properties.

(C) Expanded repeats in microsatellite diseases may lead to sequestration of RBPs into RNA foci and/or abnormal interaction with repeat-containing proteins translated from the expansion.

(D) Disruption of RBPs has a widespread effect on the metabolism of their RNA targets including abnormal splicing, polyadenylation, transport, translation, and decay with downstream effects on cellular morphology and function.

the nucleus and the final assembly of the snRNPs takes place in Cajal bodies. In SMA, abnormal snRNP assembly is either due to deletion or to missense mutations that disrupt the ability of SMN protein to oligomerize and/or to interact with Sm proteins (Lorson et al., 1998; Pellizzoni et al., 1999; Seng et al., 2015; Wan et al., 2005). Loss of SMN1 function causes tissue- and snRNP-specific changes in the snRNP repertoire that result in widespread splicing defects (Gabanella et al., 2007; Huo et al., 2014; Imlach et al., 2012; Lotti et al., 2012; Zhang et al., 2008). Indeed, loss of SMN protein preferentially reduces the accumulation of minor (U12-dependent) spliceosome (Gabanella et al., 2007; Zhang et al., 2008) and mainly affects the expression of U12-targeted introns, including in the gene Stasimon that encodes a protein required for motor circuit function (Imlach et al., 2012; Lotti et al., 2012). While widespread splicing changes were observed in tissues of SMA mice (Zhang et al., 2008), they are more apparent in late-symptomatic animals; hence, it is difficult to discriminate between direct effects of SMN deficiency and consequences of neurodegenerative processes (Bäumer et al., 2009). Notably, independent from its role on splicing, U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation (Kaida et al., 2010) and promotes

Table 1. Summary of RBPs Involved in Neurological Diseases

RBP	Disease	Mechanism
Reduced RBP Expression		
Survival of motor neuron 1 and 2 (SMN1 and SMN2)	spinal muscular atrophy (SMA)	loss-of-function mutations, homozygous deletions
Fragile X mental retardation protein (FMRP)	fragile X syndrome (FXS)	CGG > $_{200}$ repeat expansion in the 5′ UTR of *FMR1*
Fox-1 homolog (RBFOX1)/ataxin 2-binding protein 1 (A2BP1)	autism spectrum disorder (ASD), intellectual disability, epilepsy	chromosomal translocations, copy number variations, mutations
Senataxin (SETX)	ataxia with oculomotor apraxia type 2 (AOA2), amyotrophic lateral sclerosis 4 (ALS4)	recessive mutations (AO2), dominant mutations (ALS4)
Angiogenin (ANG)	amyotrophic lateral sclerosis (ALS)	mutations
Neuro-oncological ventral antigen 1/2 (NOVA1/2)	paraneoplastic opsoclonus-myoclonus ataxia (POMA)	autoantibodies
Hu proteins (HuB, HuC, HuD)	paraneoplastic sensory neuronopathy and/or paraneoplastic encephalomyelitis (PSN-PEM)	autoantibodies
Aggregation-Prone RBPs		
TAR DNA binding protein 43 (TDP-43)	ALS, frontotemporal dementia (FTD)	mutations, mislocalization
FET proteins (fused in sarcoma/translocated in liposarcoma, FUS/TLS; Ewing's sarcoma, EWS; TATA-binding protein-associated factor 15, TAF-15)	ALS, FTD	mutations, mislocalization, aberrant methylation
Heterogeneous ribonucleoparticle proteins (hnRNPA2B1, hnRNPA1)	ALS, FTD	mutations
T cell-restricted intracellular antigen-1 (TIA1)	ALS, FTD	mutations
Matrin 3 (MATR3)	ALS	mutations
Ataxin 2 (ATXN2)	spinocerebellar ataxia type 2 (SCA2), ALS	$CAG_{27–33}$ (ALS) or CAG > $_{33}$ (SCA2) repeat expansion in the coding sequence of *ATXN2*
RBP Sequestration by Expanded RNA and Proteins		
DM1 protein kinase (DMPK)	myotonic dystrophy type 1 (DM1)	$CTG_{50–3,500}$ repeat expansion in the 3′ UTR of *DMPK* sequesters MBNL
Zinc finger 9 (ZFN9)	myotonic dystrophy type 2 (DM2)	$CCTG_{75–11,000}$ repeat expansion in intron 1 of *ZFN9* sequesters MBNL
C9ORF72	ALS, FTD	(G_4C_2) > $_{30}$ repeat expansion in intron 1 of *C9ORF72*. Both expanded transcripts and dipeptide repeat proteins interact with RBPs
FMRP	fragile x-associated tremor/ataxia syndrome (FXTAS)	$CGG_{55–200}$ repeat expansion in the 5′ UTR of *FMR1* recruits Pur-α and hnRNPA2/B1

transcription elongation in the sense-coding direction, highlighting how loss of SMN and alteration of snRNP assembly may impact diverse steps of RNA processing (Almada et al., 2013). Several lines of evidence also support a general role of SMN as a chaperone that promotes the assembly of various RNP complexes and influences several aspects of RNA metabolism including the 3′ processing of histone mRNAs (Tisdale et al., 2013) and mRNA decay and transport (Li et al., 2014). In neurons, SMN and a subset of its gemin partners localize to dendrites and axons (Carrel et al., 2006; Pagliardini et al., 2000; Todd et al., 2010; Zhang et al., 2006), contributing to neuronal mRNA trafficking and local translation of specific transcripts including β-actin mRNA (Jablonka et al., 2007; Kye et al., 2014; Rathod et al., 2012; Rossoll et al., 2002; Zhang et al., 2006). Consistently, SMN-deficient motor neurons exhibit reduced neurite length and altered growth cones (Akten et al., 2011; Fallini et al., 2011; Rossoll et al., 2003).

A striking convergence between SMA and amyotrophic lateral sclerosis (ALS), another motor neuron disease, has been uncovered. Indeed, SMN-Gemins complexes cluster into membraneless nuclear structures called gems (gemini of Cajal bodies) (Liu and Dreyfuss, 1996) that are lost in cells of SMA patients. Surprisingly, the number of gems is also reduced in cells of ALS patients (Sun et al., 2015; Yamashita et al., 2013; Yamazaki et al., 2012; Yu et al., 2015) and various ALS mouse models expressing mutant SOD1, FUS, or TDP-43 (Gertz et al., 2012; Ishihara et al., 2013; Kariya et al., 2012; Shan et al., 2010; Sun et al., 2015). Notably, increasing neuronal SMN levels extended lifespan and attenuated neurodegeneration in mutant TDP-43 mice (Perera et al., 2016) and improved survival of induced

pluripotent stem cell (iPSC)-derived motor neurons with TDP-43 and SOD1 mutations (Rodriguez-Muela et al., 2017). In addition, SMN and specific proteins from the U1 snRNP complexes were shown to directly interact with FUS, an RBP associated with ALS and frontotemporal dementia (FTD) (Gerbino et al., 2013; Groen et al., 2013; Sun et al., 2015; Yamazaki et al., 2012; Yu et al., 2015). The U1 snRNA is directly bound by FUS (Lagier-Tourenne et al., 2012) and is redistributed to the cytoplasm (Gerbino et al., 2013; Yu et al., 2015) along with the SmB/B′ proteins (Yu et al., 2015) in the presence of ALS-associated FUS mutations. The mechanisms leading to a reduced number of nuclear gems in different forms of ALS are not elucidated but could result from the abnormal cytoplasmic interactions of SMN with ALS-related aggregation-prone proteins. The association of SMN and ALS is further supported by human genetic studies; specifically, aberrant SMN1 copy number appears to be associated with an increased risk of ALS (Corcia et al., 2009). While SMN dysfunction emerges as a common feature in SMA and ALS, the exact mechanisms leading to motor neuron loss remain elusive. Indeed, the splicing, stability, and transport of hundreds of mRNAs are predicted to be altered by SMN dysfunction; however, the relative contribution of these events to motor neuron death is not yet established.

Reduced Expression of FMRP and RBFOX1 in Autism Spectrum Disorders

Certain autism spectrum disorders (ASDs) can be attributed to the reduced expression of RBPs including fragile X mental retardation protein (FMRP) and fox-1 homolog (RBFOX1, also known as ataxin 2-binding protein 1, A2BP1). FXS is the most common inherited form of neurodevelopmental disorder with autistic symptoms. It occurs in ~1 in 4,000 males and 1 in 8,000 females and is characterized by behavioral disorders, learning disabilities, and distinctive physical features including macroorchidism. FXS is caused by a $CGG > _{200}$ microsatellite repeat expansion in the 5′ UTR of the *FMR1* gene, which encodes FMRP (Kremer et al., 1991; Oberlé et al., 1991; Verkerk et al., 1991; Yu et al., 1991). This large CGG expansion induces the silencing of the *FMR1* gene via DNA hypermethylation, as well as histone hypoacetylation and hypermethylation (Coffee et al., 1999; Kumari et al., 2012; Oberlé et al., 1991; Sutcliffe et al., 1992; Verkerk et al., 1991), leading to the absence of FMRP in FXS patient neurons (Devys et al., 1993; Verheij et al., 1993). FMRP is necessary for the maintenance and stability of neural synapses (Coffee et al., 1999; Kumari et al., 2012; Weiler and Greenough, 1999). Indeed, neuronal dendrites from FXS patients and mouse models have abnormally long, thin, and dense spines, which correlate with immature-like morphology, supporting a role for FMRP in synaptic pruning and maturation (He and Portera-Cailliau, 2013; Irwin et al., 2001). It is noteworthy that a tight regulation of mRNA localization and spatial activation of translation is crucial to maintain neuronal integrity and synaptic function. This is in part facilitated by the formation of RNA granules composed of RNA molecules, interacting RBPs, motor proteins, and adaptor complexes that function to coordinate the transport of RNAs to specific subcellular locations (Anderson and Kedersha, 2009; Chyung et al., 2018). While in transit, translation of mRNAs is often repressed in order to ensure protein production only upon the receipt of proper signals at the appropriate location (Anderson and Kedersha, 2009). FMRP plays a critical role in synaptic plasticity and metabotropic glutamate receptor (mGluR)-dependent long-term depression by controlling the transport and local translation of specific mRNA targets in synapses (Hagerman et al., 2017; Santoro et al., 2012). In neurons FMRP interacts with kinesins including KIF3C and KIF5 to control the transport of its mRNA targets (Davidovic et al., 2007; Dictenberg et al., 2008) and localizes to the postsynaptic spaces of dendritic spines where it may function as a translational repressor influencing local protein synthesis (Feng et al., 1997; Schütt et al., 2009; Weiler et al., 1997; Zalfa et al., 2003). FMRP is present in different RNA granules, including stress granules, which form when translation initiation is impaired (Anderson and Kedersha, 2006; Antar et al., 2004; Bagni and Oostra, 2013; Dictenberg et al., 2008; Kanai et al., 2004; Kao et al., 2010; Zalfa et al., 2006). FMRP also associates with polyribosomes (Feng et al., 1997; Khandjian et al., 1996; Weiler et al., 1997), and genome-wide analyses support that FMRP binds to coding sequences of mRNAs associated with stalled ribosomes, including transcripts implicated in other ASDs (Ascano et al., 2012; Darnell and Klann, 2013; Darnell et al., 2011). However, there is also evidence that FMRP binds to non-coding regions of mRNAs and several FMRP RNA binding sites have been proposed, including G quadruplex, Kissing complex, SoSlip structure, and ACUG UGGA motifs (Ascano et al., 2012; Bechara et al., 2009; Brown et al., 2001; Darnell et al., 2001, 2005; Edbauer et al., 2010; Gessert et al., 2010; Miyashiro et al., 2003; Muddashetty and Bassell, 2009; Schaeffer et al., 2001; Suhl et al., 2014; Tian et al., 2013; Vasilyev et al., 2015). Several models for translational control by FMRP have been proposed. FMRP can prevent the tethering of the ribosome at the 5′ end of the mRNA by recruiting cytoplasmic FMRP-interacting protein 1 (CYFIP1), which prevents interaction between translation initiation factors (Napoli et al., 2008). FMRP also directly binds to the L5 protein on the 80S ribosome (Chen et al., 2014), and an alternate model is that FMRP causes ribosomes to stall during the elongation phase of translation (Ceman et al., 2003; Darnell et al., 2011; Stefani et al., 2004). However, it should be noted that direct interaction of FMRP with ribosomal subunits (Chen et al., 2014) was observed with a N-terminally truncated form of the protein in *Drosophila*, a species with only one *Fmr1* gene, while mammals also express somewhat functionally redundant paralogs *FXR1P* and *FXR2P*. In addition, FMRP was shown to recruit RNA-induced silencing complex (RISC) to mRNA targets, repressing translation through the RNA interference pathway (Caudy et al., 2002; Edbauer et al., 2010; Ishizuka et al., 2002; Jin et al., 2004; Muddashetty et al., 2011; Plante et al., 2006). Finally, an indirect mechanism was recently proposed with the identification of the diacylglycerol kinase kappa (Dgkκ) mRNA as a major target of FMRP in mouse cortical neurons (Tabet et al., 2016). Loss of FMRP is associated with decreased expression of the DGKK protein, a master regulator of lipid signaling that converts diacylglycerol (DAG) to phosphatidic acid (PA). Since downstream effectors of DAG and PA lipids influence protein translation, it was proposed that FMRP can regulate protein synthesis by an indirect (DAG-mediated) rather than a direct mechanism (Hagerman et al., 2017; Tabet et al., 2016).

In addition to mRNA transport and translation regulation, FMRP plays a role in other steps of RNA metabolism including mRNA stability (Zalfa et al., 2007), RNA editing (Shamay-Ramot et al., 2015), and the splicing of its own mRNA (Didiot et al., 2008). Although FMRP is predominantly cytoplasmic, it shuttles between the cytoplasm and the nucleus (Feng et al., 1997; Willemsen et al., 1996). Nuclear FMRP was found to associate with Cajal bodies (Dury et al., 2013) and was identified as a chromatin-binding protein that participates in the DNA damage response (Alpatov et al., 2014). In addition, FMRP interacts with other RBPs involved in neurological diseases including SMN (Piazzon et al., 2008) and TDP-43 (Majumder et al., 2016; Wang et al., 2008; Yu et al., 2012). Overall, FMRP loss may affect multiple steps of RNA processing leading to synaptic dysfunction associated with autism in FXS patients. An additional mechanism of FMRP-mediated neurodegenerative pathology involves intermediate repeat expansion in *FMR1*, which is linked to increased mRNA expression but reduced FMRP protein expression; this mechanism is discussed in detail in a subsequent section.

Reduced expression of another RBP, RBFOX1, has been associated with autism, intellectual disability, and epilepsy (Bhalla et al., 2004; Bill et al., 2013; Martin et al., 2007; Sebat et al., 2007; Voineagu et al., 2011). The predominant nuclear isoform of RBFOX1 is necessary for appropriate neuronal excitation through control of the alternative splicing of numerous transcripts, including genes associated with increased susceptibility to autism (Weyn-Vanhentenryck et al., 2014). Downregulation of RBFOX1 and aberrant splicing of putative RBFOX1 targets was identified by RNA sequencing (RNA-seq) in the cortex of autistic patients (Voineagu et al., 2011). A neural-specific knockout mouse model of RBFOX1 revealed aberrant splicing of mRNA targets involved in neuronal excitation and synaptic function (Gehman et al., 2011). Furthermore, these mice present with epilepsy, a frequent comorbidity in autistic-like traits. Besides splicing regulation in the nucleus, a cytoplasmic RBFOX1 isoform lacking the nuclear localization signal is produced from alternatively spliced transcripts. Cytoplasmic RBFOX1 mostly binds 3′ UTRs and regulates mRNA stability and translation of cortical development and autism-related genes (Lee et al., 2016a). Notably, another RBP, ELAVL2/HuB, was shown to affect the splicing and transcription of transcripts also targeted by FMRP and RBFOX1. Common targets include several synaptic proteins and ASD-associated genes (Berto et al., 2016), suggesting coordinated regulation and providing an additional candidate for ASD targeted therapeutics. ELAVL/Hu proteins are also involved in neurological diseases related to the production of autoantibodies and their roles are discussed in greater detail in the corresponding section.

Senataxin: An RNA/DNA Helicase Involved in Neurodegeneration

A severe recessive disorder called ataxia with oculomotor apraxia type 2 (AOA2) (Moreira et al., 2004) and a dominant juvenile form of ALS (ALS4) (Chen et al., 2004) are caused by mutations in the Senataxin gene (*SETX*), an RNA/DNA helicase. AOA2 is an autosomal recessive genetic disorder characterized by cerebellar atrophy, oculomotor apraxia, and axonal sensorimotor neuropathy, while ALS4 is a dominantly inherited form of juvenile ALS. Most Senataxin mutations cause premature protein termination, interfere with the function of the helicase, or affect N-terminal protein interaction domains (Chen et al., 2004; Criscuolo et al., 2006; Duquette et al., 2005; Fogel and Perlman, 2006; Moreira et al., 2004). Senataxin regulates gene expression by inducing transcriptional termination (Skourti-Stathaki et al., 2011). An RNA/DNA duplex is formed with the nascent mRNA, behind polymerase II (Pol II) and after 3′ cleavage of poly(A) sites. Senataxin acts to resolve these duplex structures, allowing access for the 5′–3′ exonuclease Xrn2 at 3′ cleavage poly(A) sites and promoting Rad51 foci formation to facilitate double-strand break repair at active genes (Cohen et al., 2018). This facilitates 3′ transcript degradation and consequent Pol II termination (Alzu et al., 2012; Skourti-Stathaki et al., 2011; Yüce and West, 2013). Another study reveals that it is the dimethylation of arginine residues in the C-terminal domain of Pol II that recruits SMN, which can then interact with senataxin to facilitate the resolution of R-loops and affect transcription termination (Zhao et al., 2016).

Angiogenin Loss of Function: A Role for tRNA Maturation in Neurodegeneration

Heterozygous missense mutations in the coding region of hypoxia-inducible factor angiogenin (*ANG*) segregate with familial and sporadic ALS, resulting in ANG loss of function (Fernández-Santiago et al., 2009; Greenway et al., 2006; Paubel et al., 2008; Wu et al., 2007; Zou et al., 2012). ANG, a member of the pancreatic ribonuclease A (RNase A) superfamily (Fett et al., 1985), is a key factor in the control of motor neuron survival by protecting against excitotoxic injury (Kieran et al., 2008). Several aspects of ANG are necessary to induce angiogenesis, the process of new blood-vessel growth, including ribonuclease activity, basement membrane degradation, signaling transduction, and nuclear translocation (Gao and Xu, 2008). In cancer cells, ANG can move into the nucleus and is able to bind to DNA and stimulate ribosomal RNA transcription (Tsuji et al., 2005). ANG is also an important component of stress-induced translational repression by inducing cleavage of tRNAs and accumulation of tRNA-derived, stress-induced small RNAs (tiRNAs) (Fu et al., 2009; Yamasaki et al., 2009). ANG mutants associated with ALS are unable to induce angiogenesis because of a deficiency in ribonuclease activity, nuclear import, and nuclear localization (Crabtree et al., 2007; Greenway et al., 2006; Wu et al., 2007).

Autoantibodies against RBPs Cause Loss-of-Function Phenotypes Associated with Neurological Disorders

Paraneoplastic opsoclonus-myoclonus ataxia (POMA) is a neurological syndrome caused by the secretion of auto-antibodies against neuro-oncological ventral antigen (Nova) 1 and 2 expressed by systemic tumors (Buckanovich et al., 1993), with an estimated incidence of 1 in 5,000,000. Nova-1 and Nova-2 are neuron-specific nuclear RBPs that are involved in the regulation of splicing (Ule et al., 2003, 2005b, 2006) and alternative polyadenylation (Licatalosi et al., 2008). Deficit of Nova proteins alters the processing of genes involved in inhibitory synaptic transmission (Dredge et al., 2005), as well as synaptogenesis (Ruggiu et al., 2009) and neuronal migration (Yano et al., 2010).

Anti-neuronal Nuclear (Hu) antibody associated paraneoplastic sensory neuronopathy and/or paraneoplastic encephalomyelitis (PSN-PEM) is an autoimmune disorder in which

patients develop symptoms of CNS dysfunction and/or sensory neuropathy not caused by metastases or other disorders (Lukacs et al., 2012; Senties-Madrid and Vega-Boada, 2001). ELAV/Hu proteins (HuB, HuC, and HuD) are a family of RNA-binding proteins implicated in neuronal differentiation and maintenance (Akamatsu et al., 1999; Anderson et al., 2000; Wakamatsu and Weston, 1997; Zhu et al., 2006). Hu proteins affect many post-transcriptional aspects of RNA metabolism, from splicing to translation. They regulate mRNA stability by interacting with AU-rich elements (AREs) in 3′ UTRs (Anderson et al., 2000; Darnell et al., 2011; Deschênes-Furry et al., 2006; Fan and Steitz, 1998; Hinman and Lou, 2008; Myer et al., 1997) and were also shown to modulate translation of mRNA targets through diverse mechanisms. Most frequently, Hu proteins were found to increase translation initiation, either by binding to the 3′ UTR and promoting stability (Antic et al., 1999; Kawai et al., 2006; Mazan-Mamczarz et al., 2003) or by binding to the 5′ UTR (Galbán et al., 2008). In the nucleus, Hu proteins interact with U-rich sequences to promote RNA stability, polyadenylation, splicing, and translation (Bellavia et al., 2007; Zhu et al., 2006, 2007, 2008).

Aggregation-Prone RBPs at the Core of Neurodegenerative Diseases

Multiple RBPs Containing Low Complexity Domains Are Mutated in ALS and FTD

Another emerging mechanism relies on the intrinsic aggregation-prone properties of several RBPs that form abnormal inclusion bodies in pathological conditions (Figure 1B). This process may result in either a gain of toxic properties, a loss of function, or both, via the RBP's cellular mislocalization and sequestration into inclusions. Disruption of RBP homeostasis has emerged as a major disease mechanism in ALS and FTD, two neurodegenerative conditions with clinical, genetic, and pathological overlap. ALS, which has an incidence of 1–2 cases per 100,000 each year, is an adult onset motor neuron disease characterized by degeneration of motor neurons in the brain and spinal cord leading to progressive muscle weakness and fatal paralysis. FTD has a prevalence of approximately 20 in 100,000 and is characterized by alterations in behavior, personality, and language associated with atrophy of the frontal and temporal lobes. Motor neuron disease and cognitive deficits of variable severity can be concomitant in patients or within families indicating a spectrum of clinical phenotypes that relate to common neuropathologic lesions in ALS and FTD.

Aggregation of different RBPs, including TAR DNA binding protein 43 (TDP-43) (Arai et al., 2006; Neumann et al., 2006), the FET proteins (Fused in sarcoma/Translocated in liposarcoma, FUS/TLS; Ewing's sarcoma, EWS; and TATA-binding protein-associated factor 15, TAF-15) (Couthouis et al., 2011, 2012; Kwiatkowski et al., 2009; Vance et al., 2009), a subset of heterogeneous ribonucleoparticle proteins (hnRNPA2B1 and hnRNPA1) (Kim et al., 2013), the Matrin 3 (MATR3) (Johnson et al., 2014), and the T cell-restricted intracellular antigen-1 (TIA1) (Mackenzie et al., 2017) proteins, have been implicated in a spectrum of neurodegenerative diseases including ALS and FTD.

Initial evidence pointing to a role for RNA processing in ALS and FTD has been the groundbreaking discovery of TDP-43 as a component of cytoplasmic and ubiquitinated inclusions in neurons of patients with sporadic ALS and FTD (Arai et al., 2006; Neumann et al., 2006). This finding was rapidly followed by the identification of TDP-43 mutations as genetic causes of ALS and FTD (Lagier-Tourenne et al., 2010). TDP-43 is primarily nuclear but shuttles between the nucleus and cytoplasm and has been implicated in several steps of RNA metabolism including splicing, transport, RNA stability, and translational repression within stress granules (Alami et al., 2014; Costessi et al., 2014; Fiesel et al., 2012; Li et al., 2013; Polymenidou et al., 2011; Tollervey et al., 2011). Most TDP-43 mutations causing familial ALS cluster within the C-terminal domain, which corresponds to a glycine-rich region essential for interactions with other proteins (Lagier-Tourenne et al., 2010). The N-terminal part of TDP-43 was recently shown to mediate physiological TDP-43 oligomerization and to antagonize the formation of pathologic aggregates (Afroz et al., 2017). Notably, TDP-43 pathology is also characterized by a striking loss of nuclear staining in neurons with cytoplasmic aggregation (Neumann et al., 2006). Determining the effect of this nuclear loss on the processing of TDP-43 mRNA targets in affected neurons represents a crucial step in elucidating disease mechanisms in TDP-43 proteinopathies.

In addition to TDP-43, mutations and mislocalization of the FET family proteins (FUS/TLS, EWS and TAF15) are also associated with ALS and/or FTD. FUS/TLS is a primarily nuclear RBP involved in various aspects of the RNA life cycle, including transcription, splicing and mRNA transport (Kanai et al., 2004; Lagier-Tourenne et al., 2010), that was identified in cytoplasmic inclusions of ALS and/or FTD patient neurons distinct from TDP-43 positive inclusions (Kwiatkowski et al., 2009; Neumann et al., 2009; Vance et al., 2009). FUS/TLS harbors a N-terminal domain enriched in glutamine, glycine, serine, and tyrosine residues that contains disease-causing mutations (Lagier-Tourenne et al., 2010). However, the majority of ALS and FTD-associated mutations are found in a C-terminal, non-canonical nuclear localization signal (NLS). Mutations in the NLS impair appropriate nuclear targeting (Dormann et al., 2010), likely contributing to subsequent cytoplasmic aggregation. Nuclear import of FUS is also influenced by abnormal arginine methylation of the protein in ALS and FTD (Dormann et al., 2010; Suárez-Calvet et al., 2016; Tradewell et al., 2012). Similarly, dominant mutations in both TAF15 and EWS have been identified in ALS and/or FTD patients (Couthouis et al., 2011, 2012), and both proteins were found in cytoplasmic aggregates in sporadic ALS (Couthouis et al., 2011, 2012) and FUS-positive sporadic FTD patients (Neumann et al., 2011).

Two members of the family of hnRNPs, hnRNPA2/B1, and hnRNPA1 were associated with a multisystemic disorder that includes ALS and FTD (Kim et al., 2013). Mutations in the C-terminal glycine-rich region of the proteins were identified in families with ALS or with a condition called multisystem proteinopathy (MSP) that includes inclusion body myopathy, Paget's disease of the bone, FTD, and ALS (Kim et al., 2013). Finally, ALS-causing mutations were identified in the gene encoding MATR3 (Johnson et al., 2014), an RBP interacting with TDP-43 (Ling et al., 2010), and involved in RNA splicing (Coelho et al., 2015), export

(Boehringer et al., 2017), and stability (Salton et al., 2011), and in the gene encoding Tia1 (Mackenzie et al., 2017), a major component of stress granules (Gilks et al., 2004; Kedersha et al., 2000).

A unifying feature shared by RBPs associated with neurodegenerative diseases is the presence of prion-like domains (PrLDs) that promote the formation of self-nucleating aggregates (March et al., 2016). PrLDs are characterized by low complexity sequences rather than a specific primary sequence and are enriched in polar, uncharged amino acids such as glutamine, asparagine, tyrosine, serine, and glycine (Alberti et al., 2009; Ross et al., 2005; Toombs et al., 2010; Wang et al., 2018). Such low complexity domains (LCDs) composed of only a few amino acids were predicted in more than 200 proteins in the human genome (Lancaster et al., 2014) with a clear enrichment for RNA and DNA binding proteins. These domains appear to be crucial for the dynamic assembly and disassembly of ribonucleoprotein granules (RNPs) (Gitler and Shorter, 2011; Kim et al., 2013; King et al., 2012; Li et al., 2013), as well as to confer the intrinsically aggregation-prone property of several RBPs associated with neurodegenerative diseases.

Indeed, low-complexity domains were recently shown to enable liquid-liquid phase separation, a process crucial for the dynamic formation of supramolecular assemblies constituting membraneless cellular compartments (Lin et al., 2015; Molliex et al., 2015; Nott et al., 2015; Patel et al., 2015). The ability of RBPs to undergo phase separation is intimately linked to their dynamic association with various nuclear and cytoplasmic membraneless organelles, including the nucleolus, nuclear speckles, Cajal bodies, nuclear pores, cytoplasmic stress granules, and P-bodies (Brangwynne et al., 2009; Frey et al., 2006; Hyman et al., 2014; Jain et al., 2016; Li et al., 2012). Failure to maintain liquid phase homeostasis has been shown to trigger fibrillization of RBPs and is proposed to be at the root of abnormal inclusions observed in neurodegenerative diseases including ALS, FTD, and Alzheimer's disease (March et al., 2016; Boeynaems et al., 2018; Burke et al., 2015; Ambadipudi et al., 2017; Conicella et al., 2016; Han et al., 2012; Hernández-Vega et al., 2017; Kato et al., 2012; Mackenzie et al., 2017; Mateju et al., 2017; Molliex et al., 2015; Monahan et al., 2017; Murakami et al., 2015; Murray et al., 2017; Patel et al., 2015; Xiang et al., 2015). Phase transition behavior from solute to liquid-like, gel-like, and solid states is influenced by various factors including temperature, local protein and RNA concentrations, or the amino acid composition and post-translational modifications of the LCDs (Boeynaems et al., 2018; Maharana et al., 2018; Wang et al., 2018). Mutations associated with neurodegeneration largely cluster within low complexity domains in the N-terminal domain of FUS, TAF15, and EWS, and in the C-terminal of TDP-43, hnRNPA2/B1, hnRNPA1, and Tia1. Disease-associated mutations in the LCDs alter the physicochemical properties of RBPs with respect to phase separation leading to a faster and sometimes irreversible transition of RBPs from liquid droplets into less dynamic structures such as hydrogels and eventually fibrillar solids reminiscent of the aggregations observed in post-mortem human CNS tissues (Kim et al., 2013). Notably, increasing the cytoplasmic concentration of RBPs results in liquid-liquid phase separation and increased assembly of stress granules (Guo et al., 2018; Kim et al., 2013; Lin et al., 2015; Mackenzie et al., 2017; Molliex et al., 2015; Murakami et al., 2015; Patel et al., 2015). Hence, while ALS and/or FTD mutations in the NLS of FUS do not directly alter the propensity of FUS to aggregate (Sun et al., 2011), they impair nuclear import of FUS leading to higher cytoplasmic concentration and phase separation of the protein into FUS droplets that mature to more solid aggregates.

While it appears that RBPs are prone to aggregation and may play a role in disease propagation due to PrLDs (Polymenidou and Cleveland, 2011), a great deal remains unknown about the interplay between the proteins, the mechanistic role of the various mutations in each of the proteins, and the potential for aggregate gain of function.

Genome-wide Studies Reveal Complex Networks between Disease-Associated RBPs and Their RNA Targets

Notably, RBP levels are tightly regulated and genome-wide analyses of binding sites by cross-linking immunoprecipitation (CLIP) approaches (Licatalosi et al., 2008; Ule et al., 2005a) have uncovered complex regulatory mechanisms between different RBPs (Huelga et al., 2012; Lagier-Tourenne et al., 2012; Martinez et al., 2016; Polymenidou et al., 2011; Rogelj et al., 2012; Sanford et al., 2009; Tollervey et al., 2011; Yeo et al., 2009). Indeed, RBPs often bind their own RNAs, as well as RNAs encoding other RBPs, generating feedback loops crucial for RNA homeostasis. For example, both TDP-43 and FUS/TLS bind their own mRNAs and induce dose-dependent splicing alterations that produce transcripts subjected to nonsense-mediated decay as part of complex auto-regulatory mechanisms (Avendaño-Vázquez et al., 2012; Ayala et al., 2011; Bembich et al., 2014; D'Alton et al., 2015; Lagier-Tourenne et al., 2012; Polymenidou et al., 2011; Zhou et al., 2013). ALS-associated mutations in FUS/TLS were shown to compromise this auto-regulatory loop and may participate in a feedforward mechanism enhancing FUS/TLS aggregation in affected neurons (Zhou et al., 2013). Notably, increasing the amount of human up-frameshift protein 1 (hUPF1), an ATP-dependent RNA helicase and central nonsense-mediated decay factor, was shown to rescue toxicity associated with both FUS and TDP-43 overexpression (Barmada et al., 2015; Ju et al., 2011). In addition, intertwined relationships between different aggregation-prone RBPs have been observed, including decreased levels of FUS transcript upon TDP-43 knockdown (Polymenidou et al., 2011), or elevated TAF15 levels upon FUS reduction (Lagier-Tourenne et al., 2012). Consequently, disruption of one RBP may induce widespread alterations of RNA processing engaging direct RNA targets as well as transcripts not bound by the primary altered RBP.

Systematic analyses of the RNA targets of aggregation-prone RBPs, in particular, comparisons of the binding patterns between TDP-43 and FUS/TLS, the members of the FET family (FUS/TLS-EWSR1-TAF15), Tia1, or the hnRNP family have revealed points of convergence but also striking differences in the role of each RBP (Alarcón et al., 2015; Blechingberg et al., 2012; Colombrita et al., 2012; Goodarzi et al., 2012; Hoell et al., 2011; Huelga et al., 2012; Ibrahim et al., 2013; Ishigaki et al., 2012; Kapeli et al., 2016; Lagier-Tourenne et al., 2012; Martinez et al., 2016; Meyer et al., 2018; Nakaya et al., 2013; Polymenidou et al., 2011; Rogelj et al., 2012; Sephton et al., 2011;

Tollervey et al., 2011; Vogler et al., 2018; Xiao et al., 2011). Although genome-wide analyses established that TDP-43 and FUS/TLS have mostly distinct functions in regulating the processing of their targets, an unexpected role was uncovered in sustaining the levels of mRNAs transcribed from genes with exceptionally long introns that encode proteins essential for neuronal function (Lagier-Tourenne et al., 2012; Polymenidou et al., 2011). A subset of these proteins was also found to be reduced in TDP-43 aggregate-containing motor neurons in sporadic ALS, supporting a common loss-of-function pathway as one component underlying motor neuron death in TDP-43 and FUS/TLS proteinopathies (Lagier-Tourenne et al., 2012). In addition, several hundred splicing alterations were identified upon reduction of aggregation-prone RBPs (Ibrahim et al., 2013; Ishigaki et al., 2012; Lagier-Tourenne et al., 2012; Martinez et al., 2016; Paronetto et al., 2011; Polymenidou et al., 2011; Rogelj et al., 2012; Tollervey et al., 2011). Only a subset of TDP-43-regulated exons identified by RNA-seq had previous expressed sequence tag (EST)/mRNA evidence for alternative splicing, demonstrating a role for TDP-43 to regulate previously unknown alternative splicing events (Polymenidou et al., 2011). Among non-annotated aberrant splicing events, approximately 50 exons were found to be included upon TDP-43 depletion, consistent with a normal role for TDP-43 in repressing the inclusion of cryptic exons (Ling et al., 2015; Tan et al., 2016). Abnormal inclusion of cryptic exons (Ling et al., 2015) along with aberrant splicing of the *SORTILIN 1* and *POLDIP3* transcripts (Prudencio et al., 2012; Shiga et al., 2012) observed in brain tissues of ALS and/or FTD patients with TDP-43 pathology strongly support a loss-of-function mechanism in TDP-43 proteinopathies. Among thousands of TDP-43-mediated RNA alterations, human stathmin-2 was recently shown to be the most affect transcript upon TDP-43 loss (Klim et al., 2019; Melamed et al., 2019). Aberrant splicing and premature polyadenylation lead to accumulation of a truncated transcript in affected tissues from sporadic and C9ORF72-related ALS patients and restoration of stathmin-2 levels was shown to be crucial for axonal regeneration upon TDP-43 loss. In addition, FUS was shown to mediate the interaction between RNA polymerase II and U1 snRNP, hence coupling transcription to splicing (Sun et al., 2015; Yu et al., 2015). Besides alternative splicing, both TDP-43 and FUS/TLS were proposed to influence the use of alternative polyadenylation sites (Masuda et al., 2015; Rot et al., 2017). Genomic approaches also provided insights on the RNA binding sites and roles on alternative splicing and polyadenylation of hnRNPA2B1 (Alarcón et al., 2015; Goodarzi et al., 2012; Martinez et al., 2016) and Tia1 (Meyer et al., 2018). Notably, hnRNPA2B1 was found to bind m^6A-bearing RNAs and elicit similar alternative splicing effects as the m^6A writer METTL3 (Alarcón et al., 2015). Finally, TDP-43, FUS and hnRNPA2B1 were shown to bind microRNAs and modulate microRNA biogenesis with broad implications on RNA biology (Alarcón et al., 2015; Eitan and Hornstein, 2016). Overall, genomic approaches have uncovered widespread RNA processing alterations in aggregation-prone neurodegenerative diseases. Identification of splicing and/or expression alterations that directly contribute to neuronal dysfunction may have major implications for therapeutic development in ALS and FTD.

The Cytoplasmic Roles of RBPs Are Also Altered in ALS and FTD

It is noteworthy that alterations of an RBP's cytoplasmic functions may play a role in neurodegeneration along with loss of nuclear functions in affected neurons. Indeed, aggregation-prone RBPs involved in ALS and/or FTD such as TDP-43, FUS/TLS, TAF-15, EWSR1, hnRNPA1, and hnRNPA2/B1 have been implicated in RNA transport and local translation of their RNA targets (Alami et al., 2014; Andersson et al., 2008; Jean-Philippe et al., 2013; Lagier-Tourenne et al., 2010). Notably, the cytoplasmic function of TDP-43 in delivering mRNA targets to distal neuronal compartments is impaired in the presence of ALS-related mutations (Alami et al., 2014). Comparative CLIP analysis of wild-type FET proteins (FUS, EWSR1, and TAF15) and ALS mutant FUS/TLS has also revealed that mutant FUS/TLS binds more cytoplasmic targets, as demonstrated by increased fraction of binding on mature RNAs (UTRs and coding regions), consistent with the cytoplasmic mislocalization of mutant FUS/TLS (Hoell et al., 2011). A role of the FET proteins in mRNA transport has been proposed (Andersson et al., 2008), but it is still unknown whether ALS-linked mutations disrupt the transport of specific mRNA targets. However, deficient axonal transport of mitochondria and lysosomes was recently identified in iPSC-derived motor neurons with FUS mutations (Guo et al., 2017; Naumann et al., 2018). Expression of human mutant FUS in transgenic mice was also shown to activate an integrated stress response and to inhibit intra-axonal protein synthesis in hippocampal neurons and sciatic nerves (Lopez-Erauskin et al., 2018).

Finally, aggregation-prone RBPs involved in neurodegeneration often co-localize with markers of stress granules. Stress granules are dynamic, self-assembling, membraneless structures that contain translationally arrested mRNA-protein complexes, and usually form in the presence of cellular stress. They are believed to function as a triage unit to determine whether mRNAs should be degraded, released for translation, or remain sequestered and translationally repressed (Protter and Parker, 2016). As discussed above, unbalanced assembly and disassembly of stress granules through altered phase separation homeostasis is likely to play a role in the emergence of cytoplasmic inclusions observed in patients (Bosco et al., 2010; Li et al., 2013; Mackenzie et al., 2017; Mitchell and Parker, 2014; Murakami et al., 2015; Patel et al., 2015; Ramaswami et al., 2013; Wolozin, 2012). The implementation of enzymatic-based biotin proximity labeling identified hundreds of known and previously unknown stress granule-associated proteins, and it was shown *in vivo* that a subset of these stress granule-associated RBPs is capable of modulating the toxicity driven by mutant ALS-associated RBPs FUS and TDP-43 (Markmiller et al., 2018). The RBP TIA1 was also shown to interact with tau to promote tau misfolding, leading to protein aggregation and formation of stress granules both *in vitro* (Vanderweyde et al., 2016) and *in vivo* (Apicco et al., 2018). Finally, besides the cytoplasmic role of aggregation-prone RBPs in stress granules, TDP-43 was recently shown to play a functional role in muscle formation and regeneration through the constitution of amyloid-like oligomeric assemblies called "myo-granules" which are cleared as myofibers mature (Vogler et al., 2018).

Aggregation of RBPs in Polyglutamine Expansion Diseases

CAG trinucleotide repeat expansions encoding for long polyglutamine (polyQ) stretches are associated with several neurodegenerative diseases including Huntington's disease (a progressive disorder resulting in impaired cognition, psychiatric manifestations and abnormal movements called chorea) and several spinocerebellar ataxias (SCAs). It is noteworthy that polyQ-containing proteins were shown to sequester different RBPs containing LCDs (Figure 1C). In particular, FUS was identified by a proteomic approach as a major component of the intranuclear polyQ aggregates in cellular models of Huntington's disease and SCA3 (Doi et al., 2008) and found to colocalize with aggregates in brains from mice and patients (Doi et al., 2008, 2010). Sequestration of TDP-43 dependent on its C-terminal LCD was also observed in cellular models overexpressing polyQ (Fuentealba et al., 2010). However, it is still not established whether altered processing of TDP-43 and FUS RNA targets contribute to the pathogenesis of polyQ neurodegenerative diseases.

Besides the indirect effect of polyQ stretches in sequestering RBPs, trinucleotide (CAG) repeats within two RBPs, ataxin 1 and ataxin 2, cause SCA types 1 and 2 (SCA1 and SCA2). SCA2 is triggered by an expansion of more than 33 repeats and is characterized by a progressive cerebellar ataxia leading to abnormal balance, eye movements, and speech, often associated with dystonia, chorea, or dementia. In addition to SCA2, intermediate-length polyQ expansions (27–33 glutamines) in the *ATXN2* gene are associated with an increased risk of ALS (Elden et al., 2010). Ataxin 2 interacts with the poly(A)-binding protein (PABP) and DEAD/H-box RNA helicase (DDX6) (Ciosk et al., 2004; Hua and Zhou, 2004; Nonhoff et al., 2007; Ralser et al., 2005), two components of stress granules and P-bodies, which are stress-induced cytoplasmic foci that have a role in RNA degradation and may dock with stress granules to receive RNAs for turnover (Kedersha et al., 2005). Alteration of ataxin 2 levels interferes with the assembly of P-bodies and stress granules, and ataxin 2 is required for miRNA-mediated repression of several translational reporters *in vivo* (McCann et al., 2011; Nonhoff et al., 2007). Direct binding of ataxin 2 to RNAs was recently demonstrated using a CLIP approach (Yokoshi et al., 2014). Ataxin 2 binds a uridine-rich element within the 3′ UTR of its targets in a PABP1-independent manner and likely plays a role in the regulation of polyadenylation and stabilization of its mRNA targets (Ostrowski et al., 2017; Yokoshi et al., 2014). In addition, ataxin 2 was shown to assemble with polyribosomes (Satterfield and Pallanck, 2006) and global reduction of protein synthesis in Atxn2 knockout mice suggest that ataxin 2 promotes general translation (Fittschen et al., 2015). Finally, ataxin 2 was shown to preserve genome integrity by repressing the deleterious accumulation of RNA-DNA R-loop structures within the nucleus (Salvi et al., 2014). The exact mechanisms leading to either SCA2 or ALS are not well understood; however, ataxin 2 protein was identified as a major modulator of TDP-43 toxicity. Indeed, ataxin 2 directly interacts with TDP-43 and its reduction suppresses TDP-43 toxicity in various models by affecting its recruitment to stress granules and aggregation propensity (Becker et al., 2017; Elden et al., 2010; Hart and Gitler, 2012). Similarly, ataxin 2 interacts with FUS and was identified as a modifier of FUS pathology (Farg et al., 2013; Nihei et al., 2012). The intertwined relationship between ataxin 2 and other ALS-associated RBPs, including the striking repression of TDP-43 toxicity when lowering ataxin 2 levels in various animal models, designates ataxin 2 as a promising therapeutic target in ALS (Becker et al., 2017; Elden et al., 2010).

RBP Sequestration by Expanded RNAs and Proteins

RNA-Mediated Toxicity: An Alternative Path to Disrupt RBPs

Advances in our understanding of several neurodegenerative disorders have brought RBPs into the spotlight as having key roles in the development of pathology. The examples described above involve mutations within genes encoding RBPs; however, an alternative mechanism leading to the functional disruption of RBPs has emerged in diseases linked to microsatellite repeat expansions (Figure 1C). This mechanism, called RNA-mediated toxicity, involves sequestration of RBPs through binding to transcripts that contain hundreds or thousands of repeats, rendering the RBP unavailable for other substrates. Various RBPs have been implicated as essential contributors to such expansion-mediated pathology. Myotonic dystrophy (dystrophia myotonica, DM), ALS, FTD, and fragile X tremor and ataxia syndrome (FXTAS) are all characterized by genomic repeat expansions that, when transcribed into RNA, can sequester RBPs. This may occur in a stoichiometric fashion in which expansion of the repeat creates an excess of RBP binding sites. Alternatively, an RBP may have a greater affinity for either the primary or secondary structure of the repeat expansion. In both cases, the RBP is prevented from interacting with its endogenous targets, causing a multitude of biological effects that ultimately contribute to pathology.

RNA Toxicity in Myotonic Dystrophies

Myotonic dystrophies types 1 and 2 (DM1 and DM2) are inherited multisystemic disorders affecting ~1 in 8,000 individuals. Adult forms are characterized by muscular weakness and delayed relaxation of skeletal muscles (myotonia) and may include cataracts, learning disability, hypersomnia, insulin resistance, and heart conduction defects. Congenital forms of DM1 result in severe neonatal hypotonia and breathing defects associated with cognitive disabilities. In both DM1 and DM2 a genomic repeat expansion results in the accumulation of repeat-containing transcripts that aberrantly sequester RBPs. DM1 is caused by a $(CTG)_{50\ >\ 3,500}$ repeat expansion in the 3′ UTR of the *DMPK* gene (Brook et al., 1992), while a repeat expansion of $(CCTG)_{75\ >\ 11,000}$ located in intron 1 of the *ZNF9* gene causes DM2 (Liquori et al., 2001). Transcripts containing expanded (CTG) or (CCTG) repeats sequester and compromise the functions of RBPs from the muscle blind like protein family (MBNL1, 2, and 3) (Mankodi et al., 2000; Wang et al., 2012a). MBNL1 is a splicing regulator that co-localizes to CUG expansion-containing nuclear RNA foci (Jiang et al., 2004), and *in vivo* overexpression of MBNL1 efficiently reverses the DM1 myotonia phenotype (Kanadia et al., 2006). More specifically, it has been shown that sequestration of MBNL1 results in the aberrant splicing of several genes, a subset of which is directly involved in muscle function and the DM1 phenotype (Freyermuth et al.,

2016; Goodwin et al., 2015; Lee and Cooper, 2009). In addition to MBNL1 disruption, steady-state levels of CUGBP1, another splicing regulator, increase due to protein kinase C-mediated hyperphosphorylation in response to the expression of repeat expansions (Kuyumcu-Martinez et al., 2007). Notably, functional disruption of another member of the muscle blind like family, MBNL2, was shown to account for the neurological features (learning difficulties, daytime sleepiness) observed in DM1 patients (Charizanis et al., 2012). Depletion of MBNL3 was also shown to cause defects in muscle regeneration and a spectrum of DM1 age-associated pathologies including abnormal glucose metabolism in knockout mice (Choi et al., 2016; Poulos et al., 2013). Disruption of MBNL proteins in DM1 tissues leads to the re-emergence of developmentally immature alternative splicing. In addition to their role in alternative splicing, MBNL proteins have also been shown to bind the 3′ UTRs of their targets, affecting polyadenylation, RNA localization and turnover (Goodwin et al., 2015; Masuda et al., 2012; Wang et al., 2012a). Depletion of MBNL proteins led to misregulated polyadenylation, which was also observed in mouse models expressing CUG repeats and in muscles and brains from DM1 patients (Batra et al., 2014; Goodwin et al., 2015).

RNA Toxicity in ALS and FTD Linked to C9orf72

A hexanucleotide (G_4C_2) repeat expansion in the *C9ORF72* gene represents the most frequent genetic cause of ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011), with patients exhibiting hundreds to thousands of repeats compared to less than 30 in healthy individuals. At present, it is unresolved as to whether neurodegeneration is due to a loss of C9ORF72 function, a gain of toxic function or a combination of the two. However, accumulating evidence supports an RNA toxicity mechanism as a key mediator of pathogenesis. Indeed, transcripts containing the G_4C_2 repeat expansion have been shown to accumulate into nuclear RNA foci similar to those observed in myotonic dystrophy (DeJesus-Hernandez et al., 2011; Gendron et al., 2014). As in other microsatellite diseases, foci containing C_4G_2 expanded RNAs transcribed from the antisense strand of the *C9ORF72* locus have also been identified in cells and tissues from C9ORF72 ALS and/or FTD patients (Gendron et al., 2013; Lagier-Tourenne et al., 2013; Mizielinska et al., 2013; Mori et al., 2013a; Zu et al., 2013). A flurry of reports have proposed several candidate RBPs that may interact with *C9ORF72* hexanucleotide repeats *in vitro* (Cooper-Knock et al., 2014; Mori et al., 2013b; Reddy et al., 2013; Xu et al., 2013), including hnRNP H1/F (Conlon et al., 2016), ALYREF, SRSF2 (Cooper-Knock et al., 2014; Lee et al., 2013), hnRNPA1 (Sareen et al., 2013), hnRNP A3 (Mori et al., 2013b), ADARB2 (Donnelly et al., 2013), Pur-α (Sareen et al., 2013), and Nucleolin (Haeusler et al., 2014), that were found to partially co-localize with sense strand RNA foci. Transcriptome profiles in cells and brains from C9ORF72 ALS patients were shown to be distinct from sporadic ALS (Conlon et al., 2016; Cooper-Knock et al., 2015; Donnelly et al., 2013; Lagier-Tourenne et al., 2013; Prudencio et al., 2015; Sareen et al., 2013); however, it has not yet been established which RBP(s) is/are functionally disrupted by sequestration in RNA foci. Only hnRNP H-dependent splicing alterations have been reported in cells and tissues expressing G_4C_2 expansions (Conlon et al., 2016; Lee et al., 2013). Importantly, the capacity for the antisense RNA C_4G_2 repeat expansion to sequester RBPs requires further investigation and there is no consensus yet whether one or several RBPs are functionally disrupted in C9ORF72 disease leading to misregulation of their RNA targets. While interaction between RBPs and the expanded repeat RNA has been proposed, the structural and biochemical details remain relatively unknown. It has been proposed that the guanine-rich repeats form a G-quadruplex that preferentially binds a subset of RBPs (Fratta et al., 2012; Haeusler et al., 2014; Reddy et al., 2013; Zamiri et al., 2014). It will be interesting to more thoroughly explore structural dependencies of RNA-RBP interactions on a broader scale. An additional mechanism of disrupting RNA metabolism in C9ORF72 disease and other repeat expansion disorders is called repeat-associated non-ATG-dependent (RAN) translation and is discussed in detail below.

RNA Toxicity in FXTAS

Fragile X-associated tremor/ataxia syndrome (FXTAS) is yet another neurodegenerative repeat expansion disease where an RNA-mediated toxicity has been proposed as a mechanism underlying disease pathology. FXTAS presents with movement abnormalities including intention tremor, cerebellar ataxia, or parkinsonism and, in some instances, cognitive impairment. FXTAS is characterized by a $CGG_{55\ >\ 200}$ expansion in the 5′ UTR of the *FMR1* gene (Hagerman and Hagerman, 2007; Hagerman et al., 2001; Jacquemont et al., 2003). Although larger repeat expansions cause FXS as discussed above, intermediate repeat expansions of 55–200 repeats lead to a clinically distinct neurodegenerative disorder with late-onset ataxia and tremor. Contrary to FXS, the intermediate repeat expansion in *FMR1* is associated with increased mRNA expression but reduced expression of the FMRP protein proportional to repeat length (Kenneson et al., 2001). The mechanism of pathogenesis is still debated, but there is some evidence that the repeat expansion in RNA acts to sequester RBPs. For example, both Pur-α and hnRNPA2/B1 have been reported to bind the repeat expansion in a fly model (Jin et al., 2007; Sofola et al., 2007). These RBPs typically function in mRNA localization and transport in neurons, and loss of function by sequestration could lead to a severe phenotype. Perhaps most compellingly, overexpression of Pur-α and hnRNPA2/B1 RBPs in the fly model alleviated the neurodegenerative phenotype. In addition to these RBPs, significant work demonstrated sequestration of DGCR8 and DROSHA in both human and mouse FXTAS tissues (Sellier et al., 2013). DGCR8 and DROSHA co-localize with CGG repeats, and the processing of miRNAs was found to be reduced in patient tissues, further supporting a sequestration model. In addition, while dendritic complexity and neuronal cell viability was reduced in primary cultures of mouse cortical neurons expressing CGG repeats, overexpression of DGCR8 in these cells rescued both dendritic morphology and neuronal cell death. Surprisingly, Sellier et al. identified Pur-α and hnRNPA2/B1 as binding CGG repeats, but preferentially to non-pathogenic short repeats. In addition to DGCR8 and DROSHA, sequential sequestration of Sam 68, hnRNPG, and MBNL1 by CGG repeat expansions was identified and confirmed by fluorescence in situ hybridization (FISH)/immunofluorescence (IF) (Sellier et al., 2010). Loss

of Sam68, an RBP involved in regulation of alternative splicing, causes defects in motor coordination (Lukong and Richard, 2008) supporting that sequestration of Sam68 may play a significant role in the FXTAS pathology, although further transcriptome-wide studies identifying the splicing defects in FXTAS patients must be undertaken.

Sequestration of RBPs by Abnormal Proteins Translated from Expanded Repeats

As discussed above, long polyQ stretches translated from repeat expansions in coding regions were shown to directly or indirectly alter RBP homeostasis. In addition, transcripts with repeat expansions in coding and non-coding regions were shown to undergo RAN translation, producing abnormal mono or dipeptide repeat proteins (Zu et al., 2011). RAN translation occurs in the absence of an AUG start codon, in multiple reading frames of the same transcript, and within coding as well non-coding regions. This mechanism has now been described in several expansion diseases including SCA8 (Zu et al., 2011) and SCA31 (Ishiguro et al., 2017), DM1 and DM2 (Zu et al., 2017; Zu et al., 2011), Huntington's disease (Bañez-Coronel et al., 2015), FXTAS (Todd et al., 2013), and C9ORF72 ALS and/or FTD (Ash et al., 2013; Gendron et al., 2013; Mori et al., 2013a, 2013b, 2013c; Zu et al., 2013). In C9ORF72 disease, 5 different dipeptide repeat (DPR) proteins translated through RAN translation from both G_4C_2 and C_4G_2 transcripts accumulate in patients. Increasing evidence supports that DPR proteins, in particular, arginine-rich poly-GR and poly-PR proteins, are toxic and play a central role in neurodegeneration due to C9ORF72 expansions (Kwon et al., 2014; May et al., 2014; Mizielinska et al., 2014; Saberi et al., 2018; Tao et al., 2015; Wen et al., 2014; Yamakawa et al., 2015; Zhang et al., 2014). Most importantly, arginine-containing DPRs were recently shown to interact mainly with RBPs and proteins with LCDs including TDP-43, hnRNPA1, and FUS/TLS (Kanekura et al., 2016; Lee et al., 2016b; Lin et al., 2016; Lopez-Gonzalez et al., 2016). Poly-GR and poly-PR were shown to perturb the phase separation of proteins with LCDs and disrupt the assembly and function of membrane-less organelles such as stress granules, nucleoli and the nuclear pore complex (Boeynaems et al., 2017; Lee et al., 2016b; Lin et al., 2016; Shi et al., 2017). In particular, accumulation of DPRs (Boeynaems et al., 2016; Freibaum et al., 2015; Jovičić et al., 2015; Rossi et al., 2015; Zhang et al., 2015), as well as other aggregation-prone proteins such as TDP-43 (Chou et al., 2018), Huntingtin (Gasset-Rosa et al., 2017; Grima et al., 2017), and Tau (Eftekharzadeh et al., 2018), disrupts the integrity of the nuclear pores, alters nuclear-cytoplasmic transport and induces nuclear retention of RNAs. In addition, poly-GR and poly-PR may trigger splicing alterations through aberrant association with U2 snRNP (Yin et al., 2017) and impair protein translation through interaction with ribosomal proteins (Kanekura et al., 2016; Lopez-Gonzalez et al., 2016; Zhang et al., 2018).

Therapeutic Avenues for the Treatment of RBP-Mediated Neurodegeneration

Application of powerful high-throughput technologies has revealed an RBP-mediated component to several neurological diseases, and with the discovery of novel players comes the potential for new therapeutic targets.

Therapeutics that Restore RBP Expression or Function

Several therapeutic strategies have been developed to restore RBP expression. In SMA, antisense oligonucleotides (ASOs) have been designed to bind an intronic silencer and enhance inclusion of exon 7 of *SMN2* (Figure 2Ai). It was indeed shown that ASO terminal modifications can produce differential RBP recruitment to either enhance or repress exon inclusion (Rigo et al., 2012a). This approach increased SMN2 levels and improved survival and neuromuscular function in SMA mice (Hua et al., 2008, 2010, 2011; Passini et al., 2011; Rigo et al., 2012b). Most importantly, intrathecal administration of an ASO modulating SMN2 splicing (Nursinen) was shown to be beneficial in SMA patients (Chiriboga et al., 2016; Finkel et al., 2016, 2017; Mercuri et al., 2018) and represents the first FDA approved ASO-mediated treatment in neurodegenerative diseases. SMN repletion was also successfully achieved in patients using an adeno-associated virus (AAV)-mediated gene therapy approach (Figure 2Aiv) (Mendell et al., 2017), altogether demonstrating that restoration of SMN protein level is therapeutic (Groen et al., 2018). Other approaches have been investigated in preclinical studies to increase functional SMN. For example, modified ASOs were used to increase SMN2 expression in primary neurons by inhibiting the interaction between a long noncoding RNA (SMN-antisense 1) and the polycomb repressive complex 2 (PRC2), which normally repress SMN2 expression (Woo et al., 2017). In addition, splicing regulation of SMN2 exon 7 was achieved using small molecules that successfully restored full-length SMN2 protein in two mouse models through recruitment of the U1 snRNP (Figure 2Aii) (Naryshkin et al., 2014; Pinard et al., 2017; Sivaramakrishnan et al., 2017), which normally has a weak interaction with the 5′ splice site of exon 7 (Roca and Krainer, 2009). By enhancing promoter activation, histone deacetylase (HDAC) inhibitors were also shown to alleviate symptoms and increase mean survival in SMA mouse models by increasing SMN2 levels (Figure 2Aiii) (Avila et al., 2007; Lai et al., 2017; Mutsaers et al., 2011); however, survival benefit was modest when compared to ASO therapy (38 days versus 243 days). Finally, it was recently shown that administration of flunarizine in patient fibroblasts and a mouse model of SMA can facilitate localization of functional SMN into nuclear cajal bodies (Sapaly et al., 2018), facilitating proper association of RNPs and thus proper splicing. Though the exact role of SMN in cajal bodies is unknown, it is proposed that SMN acts as a chaperone for snRNPs thereby affecting the splicing repertoire.

Innovative therapeutic strategies based on the CRISPR technology are emerging, including the development of methods to increase the expression of specific proteins. In the case of FXS, dCas9-Tet1 recruitment to the repeat expansion in the 5′ UTR of FMR1 was shown to cause targeted demethylation of the repeat expansion resulting in reactivation of FMR1 expression (Figure 2Av) (Liu et al., 2018). Removal of the repeat expansion by CRISPR/Cas9 has also been shown to restore FMR1 expression in cultured cells (Xie et al., 2016). Furthermore, small molecules that recapitulate the role of RBPs whose function is lost or aberrant in the disease state show great potential as targeted therapeutics. The loss of FMRP and its role in stalling ribosomal translocation has been phenotypically reversed (Figure 2Avi), as shown by dendritic spine maturation

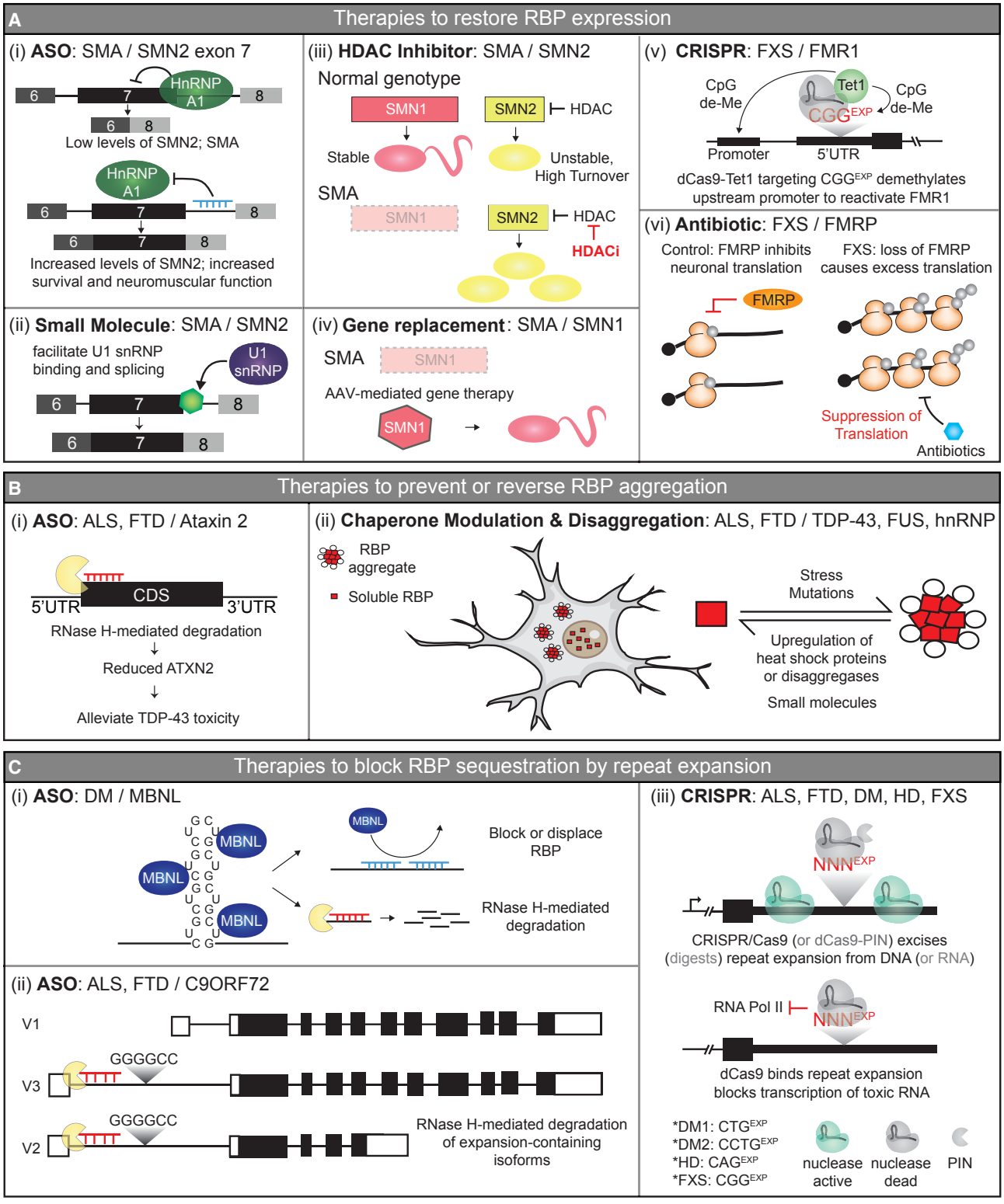

Figure 2. Therapeutic Strategies to Restore the Level and Function of RBPs in Neurodegenerative Diseases

(A) Strategies to restore RBP expression level. (i) Antisense oligonucleotides (ASOs) enhance inclusion of SMN2 exon 7 resulting in increased levels of SMN2 protein. (ii) Exon 7 inclusion in SMN2 achieved via small-molecule recruiting U1 snRNP. (iii) Restoration of SMN2 proteins levels through inhibition of Histone deacetylases (HDACs). (iv) Restoration of SMN1 protein level by AAV-mediated gene therapy. (v) Promotion of FMR1 expression using deactivated Cas9 (dCas9)

(legend continued on next page)

and restored synaptic structure, by the tetracycline analog minocycline in flies (Siller and Broadie, 2011) and mice (Bilousova et al., 2009). The antibiotic functions by occupying the A-site of the ribosome to stall translation and has been specifically shown to inhibit the overexpression of the matrix metalloproteinase-9 that occurs in *Fmr1* knockout mice (Bilousova et al., 2009). There remains some contention as to how exactly minocycline works to alleviate FXS pathology, either through global translational suppression or the suppression of specific targets; however, improvements in adolescent patient anxiety and behavior during a 3-month clinical trial (Paribello et al., 2010), as well as behavioral and cognitive improvements in adults and children (Leigh et al., 2013; Utari et al., 2010), support the need for additional characterization of the mechanism.

Therapeutics that Reverse RBP Aggregation

As discussed herein, aggregation-prone RBPs are tightly regulated and influence the processing of numerous RNA targets, precluding therapeutic strategies that reduce the overall level of the protein including ASO-mediated gene silencing. While infusion of ASOs that have specific chemical modifications leading to RNase-H mediated degradation of RNA targets holds great promise in several dominantly inherited neurodegenerative diseases (Miller et al., 2013; Schoch and Miller, 2017; Smith et al., 2006), ASO-mediated reduction of TDP-43, FUS, TAF15, or hnRNPA2B1 in the CNS of wild-type mice triggered hundreds of RNA expression and splicing alterations (Kapeli et al., 2016; Lagier-Tourenne et al., 2012; Martinez et al., 2016; Polymenidou et al., 2011). Yet, RNase-H-dependent ASOs were successfully used to target ataxin-2, a major modulator of RBP toxicity. Indeed, ASOs targeting ataxin-2 administered to the CNS of TDP-43 transgenic mice extended their survival and improved motor function, in addition to reducing aggregation of TDP-43 protein (Becker et al., 2017) (Figure 2Bi). Importantly, the approach may apply to the vast majority of sporadic ALS patients and other neurodegenerative diseases with TDP-43 proteinopathy.

In addition, efforts to modulate the misfolding and aggregation properties of RBPs have been undertaken in various models via the modulation of chaperone proteins (Figure 2Bii) (Carlomagno et al., 2014; Chen et al., 2016; Crippa et al., 2016; Gregory et al., 2012; Jackrel et al., 2014; Li et al., 2016; Park et al., 2018; Wang et al., 2017). Pharmacological upregulation of heat shock response pathways or genetic upregulation of a single heat shock protein were shown to inhibit TDP-43 aggregation. Notably, re-engineering of the yeast heat shock protein 104 (hsp104) enhanced its recognition of TDP-43 and FUS/TLS leading to significant RBP disaggregation (Jackrel et al., 2014). Most recently, nuclear import receptors were also shown to act as chaperones and potent disaggregases beyond their role in nuclear transport of RBPs (Guo et al., 2018; Hofweber et al., 2018; Qamar et al., 2018; Yoshizawa et al., 2018). Indeed, Transportin 1 (also called Karyopherin-β2) was shown to inhibit phase separation, assembly in stress granules and fibrillization of FUS/TLS (Guo et al., 2018; Hofweber et al., 2018; Qamar et al., 2018; Yoshizawa et al., 2018), TAF15, EWSR1, hnRNPA1, and hnRNPA2 (Guo et al., 2018), while Importin-α and Karyopherin-β1 prevented TDP-43 fibrilization (Guo et al., 2018). While these approaches based on modulating the level and activity of chaperones are challenging to implement in human patients, the drastic effect on aberrant phase separation merits further investigation to determine the therapeutic potential of disaggregases in neurodegenerative diseases.

Finally, small molecules can aid in the clearance of toxic accumulations of TDP-43 observed in ALS and/or FTD patients through the stimulation of autophagy (Barmada et al., 2014; Wang et al., 2012b). Inducing autophagy via small molecules improved TDP-43 clearance and mitigated toxicity in cultured neurons and astrocytes with mutant TDP-43 (Barmada et al., 2014) and improved behavior and pathology in a mouse model overexpressing TDP-43 (Wang et al., 2012b). More recently, binding of TDP-43 to poly(ADP-ribose) (PAR) was shown to promote the liquid-liquid phase transition of TDP-43, and small-molecule inhibition of the PAR polymerase Tankyrase-1/2 was found to antagonize TDP-43-associated pathology (McGurk et al., 2018). Yet another application of small-molecule therapies involves targeting the spliceosome. Though this strategy has yet to be utilized in the treatment of splicing-related neurodegenerative diseases, it has been demonstrated that the spliceosome is subject to significant modulation by small molecules, particularly via targeting of the SF3b1 subunit (Webb et al., 2013). Alternative splicing can also be modulated by small molecules targeting serine-arginine-rich proteins that bind to specific sequences called exonic splicing enhancer (ESE) and influence the choice of splice sites (Soret et al., 2005).

Therapeutics that Prevent RBP Sequestration by Repeat Expansion

ASO based therapies are now being brought to bear in several neurodegenerative disorders linked to repeat expansions either located in non-coding regions, as in myotonic dystrophy (Mulders et al., 2009; Wheeler et al., 2009, 2012; Wu et al., 2004) and C9ORF72 ALS and/or FTD (Lagier-Tourenne et al., 2013; Donnelly et al., 2013; Sareen et al., 2013; Jiang et al., 2016), or encoding for polyQ tracts as in Huntington's disease (Kordasiewicz et al., 2012) and SCAs (Moore et al., 2017; Scoles et al., 2017; Toonen et al., 2017). Depending on chemical modifications, ASOs can either bind to the repeats, alter secondary structure formation and attenuate RBP binding as shown for MBNL1 in DM1 (Wheeler et al., 2009) (Figure 2Ci), or trigger RNase H-mediated degradation of their target RNAs as demonstrated in DM1

fused to Tet1 that when targeted to CGG repeat expansion leads to demethylation of the upstream promoter and gene re-activation. (vi) The tetracycline analog minocycline blocks excessive translation caused by loss of FMRP.

(B) Strategies to prevent or reverse RBP aggregation. (i) RNase-H-dependent ASOs reducing the levels of ataxin 2 alleviate TDP-43 aggregation and toxicity. (ii) Modulation of chaperones such as heat shock proteins (hsp) and disaggregases such as nuclear import factors reverse abnormal phase transition and aggregation of RBPs.

(C) Strategies to block RBP sequestration by repeat-expanded RNAs and proteins. ASOs have been applied to alleviate RBP sequestration by repeat expanded RNAs both by blocking and displacing muscleblind proteins or by degrading the toxic RNA in myotonic dystrophy (i), ASOs inducing isoform-specific degradation of expansion-containing transcripts alleviated pathology without reducing overall levels of C9ORF72 in ALS and/or FTD (ii). CRISPR technology was applied to repeat expansion diseases to either excise the repeat expansion or to block RNA Pol II transcription in the absence of nuclease activity (iii).

(Wheeler et al., 2012) (Figure 2Ci) and C9ORF72-mediated ALS and/or FTD (Donnelly et al., 2013; Jiang et al., 2016; Lagier-Tourenne et al., 2013; Sareen et al., 2013) (Figure 2Cii). The latter is most frequently used and currently under testing in several clinical trials (Schoch and Miller, 2017). Notably, repeat expansions are often transcribed from both DNA strands and ASOs have so far mostly been developed to target expanded transcripts from the sense strand. An alternative approach targeting the transcription elongation factor Spt4/SUPT4H1 was shown to decrease production of both sense and antisense expanded transcripts in yeast, flies and C9ORF72 patient fibroblasts, resulting in a reduction of foci and DPR proteins from both strands (Kramer et al., 2016).

Beyond ASOs, aberrant interaction between RBPs and expanded RNAs can be modulated by small molecules. Modified RNA aptamer-based screens and computer-aided RNA-structure docking strategies have identified lead compounds that bind to RNA in a structure-specific manner. Compounds that bind to expanded CUG repeats were reported to block RBP binding and relieve alternative splicing defects by increasing MBNL availability in *in vitro* models of DM (Childs-Disney et al., 2013, 2014). Other small molecules function by disrupting structural motifs of mRNA necessary for RBP binding (Coonrod et al., 2013; Simone et al., 2018; Su et al., 2014). Recently, daunorubicin hydrochloride was shown to bind CUG repeats and alleviate MBNL titration in cardiomyocytes in *Drosophila* DM1 models, rescuing molecular and cardiac function associated with DM1 (Chakraborty et al., 2018).

Last, the potential therapeutic applications for CRISPR/Cas9 are rapidly increasing, and recent work suggests that this system may provide a means to specifically target toxic RNA. Batra et al. utilized an RNA-targeting Cas9 system to degrade neurodegeneration (DM, C9-ALS, and HD)-associated microsatellite repeat expansions (Batra et al., 2017). In both exogenously expressed repeat expansions as well as patient cells, targeting repeat-containing transcripts by an RNA-specific Cas9 system resulted in a reversal of several molecular phenotypes, including degradation of the RNA, foci elimination, restoration of RBP localization, reduction in toxic protein production, and correction of aberrant splicing patterns (Batra et al., 2017). Expanded DNA can also be targeted by Cas9 either to remove the expanded repeat through DNA excision (van Agtmaal et al., 2017), or by using a non-cleaving dCas9/gRNA complex that coats the repeat DNA and blocks transcription of the toxic RNA (Figure 2Ciii) (Pinto et al., 2017). While CRISPR is a highly promising targeted therapeutic, there are several issues to consider. The first is off-target effects. While specificity at the RNA-level by RNA-targeting CRISPR approaches can be measured by RNA-seq, off-target DNA modifications at the DNA-level is difficult to detect and quantify. The second issue is delivery; while AAV is a commonly utilized delivery vehicle for human therapy, its carrying capacity is limited and Cas9 is a relatively large protein. Attempts to shrink Cas9 (Batra et al., 2017), to split Cas9 (Chew et al., 2016), and to identify Cas proteins that are smaller for RNA (such as Cas13) (Cox et al., 2017; Konermann et al., 2018; Yan et al., 2018) and DNA (Harrington et al., 2018) targeting will ultimately address this issue. Finally, there is evidence suggesting that high-levels of Cas9 elicits an immune response (Chew et al., 2016), which is problematic but could potentially be circumvented by immunosuppression or possibly by manipulation to "humanize" CRISPR/Cas proteins.

Perspectives and Summary

Mutations in RBPs and RNAs have been revealed as central to the disease pathology of many neurological disorders. Pathology may arise from reduced RBP expression, aggregation of RBPs with LCDs, or sequestration of RBPs by expanded RNAs and proteins in microsatellite diseases. Separately from the mechanism, disruption of RBPs lead to major alterations in RNA homeostasis including splicing, polyadenylation, transport, translation, and decay (Figure 1). Major advances in sequencing-based technology have provided the opportunity to determine the influence of multifunctional RBPs on their RNA targets (Nussbacher et al., 2015). These studies have demonstrated that widespread alteration of multiple steps involved in RNA homeostasis is a key contributor to the molecular basis leading to neuronal dysfunction. Most importantly, multiple therapeutic approaches that restore the level and/or function of RBPs are showing great potential for clinical applications and, in some instances, are already improving disease progression in patients. However, we still lack a clear understanding of how these aberrantly expressed RBPs and RNAs contribute to selective vulnerability of neuronal cells. Additionally, the direct versus indirect changes that lead to differences in pathology remain undistinguished. Nevertheless, a great deal has been done to elucidate commonalities among phenotypes and to clarify general mechanistic observations among these diseases. Ultimately, it may be the case that despite shared global characteristics of RBP loss, aggregation, or sequestration, the underlying molecular mechanisms are distinct. This emphasizes the need for disease-specific therapeutic approaches that exploit the pioneering efforts of other fields such as AAV-mediated gene therapy or CRISPR.

ACKNOWLEDGMENTS

This work was supported by grants from the NIH (NS103172, MH107367, EY029166, HG009889, HG004659 to G.W.Y. and NS087227 to C.L.-T.), from Target ALS (to C.L.-T. and G.W.Y.), the ALS Association (to C.L.-T. and G.W.Y.), the Pape Adams Foundation, the Association Française contre les Myopathies, and ALS Finding a Cure (to C.L.-T.). R.T. was supported by a grant from the Philippe Foundation. J.K.N. is an Achievement Rewards for College Scientists Foundation Fellow and was partially supported by the Genentech Foundation fellowship and the University of California, San Diego, Cancer Training Program through an institutional training grant from the National Institute of General Medical Sciences, T32CA067754.

DECLARATION OF INTERESTS

G.W.Y. is co-founder, member of the Board of Directors, equity holder, and paid consultant for Locana and Eclipse BioInnovations. G.W.Y. is co-founder of Enzerna and ProteoNA. G.W.Y. is a paid consultant for Aquinnah Pharmaceuticals and Ionis Pharmaceuticals and scientific advisory board member to Ribometrix and the Allen Institute of Immunology. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies. The authors declare no other competing financial interests.

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